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# Assessment of SOX11 Expression in Routine Lymphoma Tissue Sections

## *Characterization of New Monoclonal Antibodies for Diagnosis of Mantle Cell Lymphoma*

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**Key Words:** SOX11, mantle cell lymphoma, monoclonal antibody, qRT-PCR

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Mantle cell lymphoma (MCL) is a B-cell neoplasm, which usually presents with advanced stage, rapid clinical progression, and a median survival of 3 to 5 years.[1] The distinction of MCL from other B-cell lymphomas, which might present with similar histologic features, such as chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, and marginal zone lymphoma, is therefore of major importance. In addition to clinical data and histomorphology, immunohistochemistry (IHC) and genetic analyses are required for a correct diagnosis. In particular, the detection of a t(11;14) translocation, which leads to the overexpression of cyclin D1, represents an indispensable diagnostic tool as it can be demonstrated in 90% of the MCLs.[1–3] Interestingly, MCLs lacking both a t(11;14) translocation and cyclin D1 expression but with a gene expression profile similar to that of cyclin D1-positive MCLs have been recognized.[3] Additional studies led to the discovery of SOX11, which is expressed in 78% to 100% of MCLs, including the cyclin D1-negative variant,[4–7] and appears to be the best discriminatory marker for the identification of a subgroup of MCL patients with clinical indolent behavior.[8,9] SOX11 represents, therefore, an important diagnostic and prognostic marker.

The *SOX11* gene, mapping at chromosome 2p25.3, encodes a transcription factor of 441 amino acids, which belongs to a family of approximately 20 genes characterized by the presence of a conserved DNA-binding high-mobility group domain. *SOX* genes are divided into 8 subgroups according to the degree of homology of the high-mobility group domain, and *SOX11*, together with *SOX4* and *SOX12*, belong to subgroup C.[10] *SOXC* genes are expressed during embryogenesis in neuronal progenitors and

in mesenchymal cells of many organs. Moreover, *SOX11* and *SOX4* are expressed in medulloblastomas and malignant gliomas.[11,12] In hemato-lymphoid tissues, *SOX4* is the only member of the *SOX* family that has been shown to play a role in the physiological B-cell and T-cell development,[13,14] whereas its expression in hemato-lymphoid neoplasms is restricted to adult T-cell leukemia/lymphoma.[15]

So far, most IHC studies aiming at characterizing the expression of *SOX11* on formalin-fixed paraffin-embedded (FFPE) tissues have been performed with polyclonal antibodies.[4–7,16] Despite their satisfactory diagnostic results in MCL, polyclonal anti-SOX11 antibodies have some limitations including certain unspecific reactions, such as cytoplasmic staining in normal tissues and in lymphomas lacking SOX11 mRNA expression,[4,5] as well as batch to batch variation in their reactivity, which represent major limiting factors. Recently, the monoclonal anti-SOX11 antibodies have been presented; however, their capacity to discriminate against SOX4 has not been tested.[17] Moreover, no study has compared IHC results with transcriptional activity for *SOX11*, so far. The aim of this study was to investigate the possible relationship existing between mRNA levels and IHC detection for SOX11, to compare the properties of the most commonly used anti-SOX11 antibodies on IHC and Western blot, and to characterize the new mouse monoclonal antibodies and to define its potential diagnostic value for MCL.

## MATERIALS AND METHODS

### Patient Samples

A large series of 209 primary lymphoid tumors diagnosed according to the last World Health Organization 2008 classification[1] was selected for the study. The diagnoses of these cases are summarized in Table 1. Samples were obtained from the tumor bank of the Department of Pathology, Hospital Clinic, University of Barcelona. The study was approved by the Ethic Committee of the Hospital Clinic of Barcelona.

### IHC and Fluorescence In Situ Hybridization Analysis

Tissue microarray sections were prepared from FFPE material derived from 153 of 209 cases selected for the study; the 57 remaining cases were examined on whole-tissue sections. Besides cyclin D1 (clone EP12; Thermo Fisher Scientific, Runcorn, UK), all cases were stained for SOX11 by means of the new mouse monoclonal anti-SOX11 antibodies anti-SOX11$^{MRQ-58}$ (Cell Marque, Rocklin, CA) and anti-SOX11$^{143}$ (AMAb90502, clone CL0143; Atlas Antibodies, Stockholm, Sweden), the latter being raised against the same peptide as is the mouse monoclonal anti-SOX11$^{142}$ (AMAb90501, clone CL0142; Atlas Antibodies).[4,6] A goat polyclonal anti-SOX11$^{sc-17347}$ (C-20, sc-17347; Santa Cruz Biotechnology Inc., Santa Cruz, CA), raised against a peptide mapping near the C-terminus,[18] was also used. For comparison purposes, 59 cases stained with the rabbit polyclonal anti-SOX11$^{HPA000536}$ (HPA000536; Atlas Antibodies) in a previous study[4] were also included. Specific conditions of use for each antibody are provided in Table 1, Supplemental Digital Content 1 (http://links.lww.com/PAS/A183).

An interphase fluorescence in situ hybridization analysis for the t(11;14) translocation using a dual-color dual-fusion probe (Vysis; Abbott Molecular, Abbot Park, IL) was performed for all 9 cyclin D1-negative MCL cases, as previously described.[19]

### Cell Lines and Western Blot Analysis

Protein extract preparations were obtained from the MCL cell line JVM2, which has been previously shown to lack SOX11 expression,[20] as well as from the nonlymphoid HEK293 cell line and 2 HEK293 cell lines stably transfected with vectors encoding human influenza hemagglutinin (HA)-tagged SOX4-HA (HEK293-SOX4-HA) and SOX11-HA proteins (HEK293-SOX11-HA), respectively.[20,21] The expression plasmids pcDNA$_3$-SOX11-HA and pcDNA$_3$-SOX4-HA were generated as previously described.[20] For Western blot analysis, 50 μg total protein extract from JVM2, 50 μg from HEK293, and 5 μg from both HEK293-SOX11-HA and HEK293-SOX4-HA were used. Four different anti-SOX11 antibodies were applied: anti-SOX11$^{143}$, anti-SOX11$^{142}$, anti-SOX11$^{MRQ-58}$, and anti-SOX11$^{sc-17347}$. A mouse monoclonal anti–glyceraldehyde 3-phosphate dehydrogenase antibody (A-3; Santa Cruz Biotechnology Inc.) served as loading control. Membranes were developed with Pierce ECL Western blot substrate (Thermo Scientific Inc., Erembodegem, Belgium) and visualized on a LAS4000 device (Fujifilm, Düsseldorf, Germany). Protein quantification was done with Image Gauge software (Fujifilm).

### *SOX11* Expression by Microarray and Quantitative Reverse Transcriptase Polymerase Chain Reaction

*SOX11* mRNA expression was investigated by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) in 23 cyclin D1-positive MCL cases and in 2 diffuse large B-cell lymphoma (DLBCL) cases, as previously described.[8,20] Briefly, total mRNA was extracted from frozen tissue samples or FFPE tissue blocks, and potential residual DNA was removed using the TURBO DNA-free kit (Applied Biosystems, Foster City, CA). The cDNA synthesis was carried out from 500 to 800 ng total mRNA, and the product was amplified and quantified using TaqMan Universal PCR Master Mix (Applied Biosystems), TaqManMGB Probes, and primers designed using Primer Express Version 2.0: *SOX11* (probe 5′-TTTTAACCACG GATAATTG-3′; forward primer 5′-CATGTAGACTAA TGCAGCCATTGG-3′; reverse primer 5′-CACGGAG CACGTGTCAATTG-3′). The cDNA was analyzed using duplicates in an ABI Prism 7900HT Fast Sequence Detection System (Applied Biosystems). Relative quantification of gene expression was performed with the $2^{-\Delta\Delta C_t}$ method using human β-glucuronidase as the endogenous control and Universal Human Reference RNA (Stratagene; Agilent Technologies, Santa Clara, CA) as the mathematical calibrator.

**TABLE 1.** IHC SOX11 Expression in a Series of Cyclin D1-positive or Cyclin D1-negative Lymphomas, Using the Mouse Monoclonal anti-SOX11$^{MRQ-58}$, Anti-SOX11$^{143}$, and the Goat Polyclonal Anti-SOX11$^{sc-17347}$ Antibody

| CCND1 | Diagnosis | Mouse Monoclonal Anti-SOX11$^{MRQ-58}$ SOX11 Positive/Total Cases (%) (n = 205) | Mouse Monoclonal Anti-SOX11$^{143}$ SOX11 Positive/Total Cases (%) (n = 209) | Goat Polyclonal Anti-SOX11$^{sc-17347}$ SOX11 Positive/Total Cases (%) (n = 177) |
|---|---|---|---|---|
| Negative | B-LBL | 0/4 | 2/4 (50) | 0/4 |
| | T-LBL | 0/4 | 4/4 (100) | 1/3 (33) |
| | CLL | 0/9 | 0/9 | 0/9 |
| | HCL | 0/3 | 0/3 | 1/3 (33) |
| | PCM/Plasmacytoma | 0/4 | 0/4 | 0/3 |
| | MZL | 0/10 | 0/10 | 0/10 |
| | FL | 0/8 | 0/8 | 0/1 |
| | MCL | 9/9 (100) | 9/9 (100) | 9/9 (100) |
| | DLBCL | 0/43 | 2/43 (5) | 1/35 (3) |
| | PBL | 0/9 | 0/9 | 0/7 |
| | PEL | 0/2 | 0/2 | 0/2 |
| | BL | 0/32 | 22/32 (69) | 6/32 (19) |
| | BCLU | 0/5 | 0/5 | 1/4 (25) |
| | T-PLL | 0/2 | 1/2 (50) | 0/2 |
| | Extranodal NK/T-cell lymphoma | 0/1 | 0/1 | 0/1 |
| | EATL II | 0/1 | 0/1 | 0/1 |
| | HSTL | 0/3 | 0/3 | 0/3 |
| | PTCL, NOS | 0/11 | 0/11 | 0/10 |
| | AITL | 0/6 | 0/6 | 0/4 |
| | ALCL ALK$^+$ | 0/3 | 0/3 | 0/3 |
| | ALCL ALK$^-$ | 0/1 | 0/1 | 0/1 |
| | NLPHL | 0/1 | 0/1 | — |
| | cHL | 0/5 | 0/5 | 0/1 |
| Positive | HCL | 0/2 | 0/2 | 0/2 |
| | PCM/Plasmacytoma | 0/4 | 0/4 | 0/4 |
| | MCL | 20/23 (87) | 20/27 (74) | 18/23 (78) |

AITL indicates angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; BCLU, B-cell lymphoma unclassified with features intermediate between DLBCL and BL; B-LBL, B-lymphoblastic lymphoma; cHL, classical Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; EATL II, enteropathy-associated T-cell lymphoma type II; FL, follicular lymphoma; HSTL, hepatosplenic T-cell lymphoma; MZL, marginal zone lymphoma; NLPHL, nodular lymphocyte predominant Hodgkin lymphoma; PBL, plasmablastic lymphoma; PCM, plasma cell myeloma; PEL, primary effusion lymphoma; PTCL NOS, peripheral T-cell lymphoma not otherwise specified; T-LBL, T-lymphoblastic lymphoma; T-PLL, T-cell prolymphocytic leukemia.

## RESULTS

### Characterization of Monoclonal SOX11 Antibodies in a Western Blot Assay

All monoclonal anti-SOX11 antibodies were initially tested in a Western blot assay, together with the previously described polyclonal anti-SOX11$^{sc-17347}$.[18] For this purpose, protein extracts of the MCL cell line JVM2, which does not express SOX11, and of the nonlymphoid HEK293, HEK293-SOX4-HA, and HEK293-SOX11-HA cell lines were used. Polyclonal anti-SOX11$^{sc-17347}$ allowed the detection of a specific band on the protein extract of the HEK293-SOX11-HA and no band on the other cell lines. Anti-SOX11$^{143}$ highlighted a strong band for HEK293-SOX11-HA and a weak band of the same size in the HEK293-SOX4-HA cell line corresponding to SOX4, whereas no band was detected for the nontransfected HEK293 (not shown) and JVM2 cell lines. Similar results were obtained with anti-SOX11$^{142}$ (Fig. 1). The anti-SOX11$^{MRQ-58}$ only highlighted the band corresponding to SOX11 for HEK293-SOX11-HA. These data suggest although anti-SOX11$^{142}$ and anti-SOX11$^{143}$ antibodies recognized overexpressed SOX4, even though to a lesser extent, the anti-SOX11$^{sc-17347}$ and anti-SOX11$^{MRQ-58}$ did not cross-react with SOX4.

Then, we compared the SOX11/SOX4 signal intensity ratios for anti-SOX11$^{143}$ and anti-SOX11$^{142}$, which were 14.2 and 3.5, respectively, indicating 4.0 times higher specificity for anti-SOX11$^{143}$. Finally, the intensity ratios for the signals of SOX11, which were normalized over the loading control glyceraldehyde 3-phosphate dehydrogenase, indicated that anti-SOX11$^{143}$ is more sensitive than anti-SOX11$^{142}$ and anti-SOX11$^{sc-17347}$ (signal intensity ratios for SOX11 anti-SOX11$^{143}$/anti-SOX11$^{142}$ was 8.1 and anti-SOX11$^{143}$/anti-SOX11$^{sc-17347}$ was 12.4).

### IHC Detection of SOX11 Correlates With mRNA Levels in MCL

The study of the protein detection on FFPE tissue and the comparison with mRNA levels of SOX11 was performed by both qRT-PCR and IHC in 23 cyclin D1-positive MCL. For IHC staining, anti-SOX11$^{143}$, anti-SOX11$^{142}$, anti-SOX11$^{sc-17347}$, and anti-SOX11$^{MRQ-58}$ were used.

In positive cases, nuclear staining with at least weak intensity was detected in most cells, whereas negative cases presented with complete absent nuclear staining. Results of the IHC and mRNA levels of *SOX11* are shown in Table 2. Briefly, qRT-PCR values for SOX11

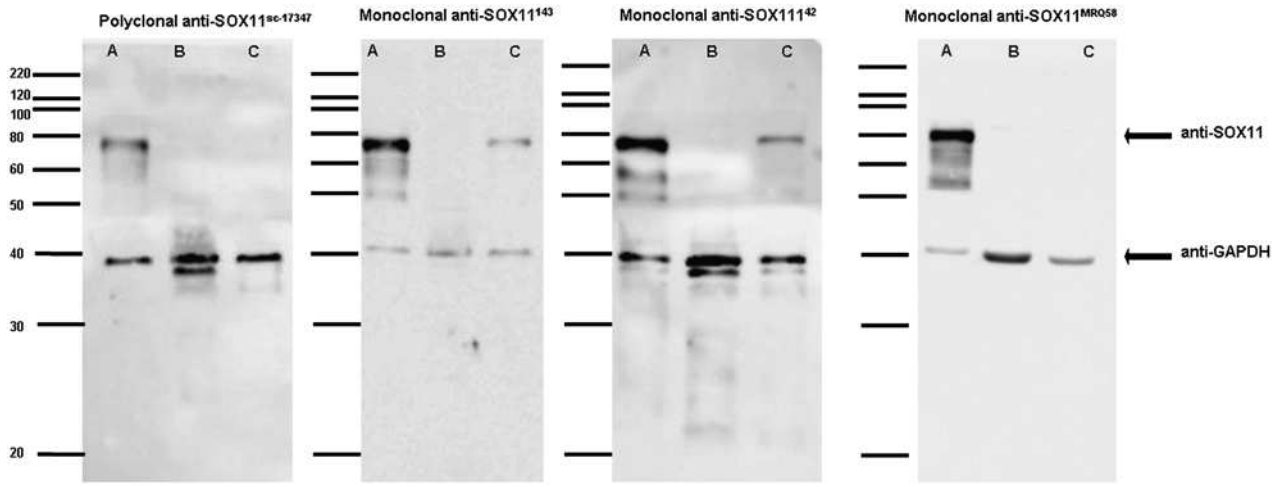


**FIGURE 1.** Western blot–based evaluation of anti-SOX11 antibodies. Western blot was performed on protein extracts derived from HEK293-SOX11-HA (A), JVM2 (B), and HEK293-SOX4-HA cell lines (C). Polyclonal anti-SOX11$^{sc\text{-}17347}$ antibody merely revealed a specific band at 60 to 80 kDa for the HEK293-SOX11-HA cell line. Monoclonal anti-SOX11$^{143}$ and anti-SOX11$^{142}$ antibodies highlighted a band of the same size for HEK293-SOX11-HA and a weaker one for HEK293-SOX4-HA, which was not detected with anti-SOX11$^{MRQ\text{-}58}$. Anti–glyceraldehyde 3-phosphate dehydrogenase antibody was used as a loading control.

ranged from 0.03 to 90.30. For all 4 antibodies, IHC for SOX11 resulted negative in cases with qRT-PCR values $\leq 1.21$ and positive for values $\geq 9.57$. Some discordance in the immunostaining with the 4 antibodies was instead observed in 2 cases with intermediate mRNA levels lying between 1.21 and 9.57 (3.53 and 3.88, respectively) (Table 2).

## Cyclin D1 and SOX11 Protein Expression in MCL and Other Lymphomas

We analyzed a series of FFPE tissues from different lymphoma subtypes and reactive lymphoid tissues to evaluate the possible use of these antibodies in routine diagnosis. Included were 9 immunophenotypically and genetically characterized cases of cyclin D1-negative MCL, which all lacked cyclin D1 expression by IHC and a t(11;14) translocation by fluorescence in situ hybridization analysis.

All positive cases exhibited distinct nuclear staining with monoclonal anti-SOX11$^{143}$ and anti-SOX11$^{MRQ\text{-}58}$, and a more heterogenous expression was observed with polyclonal anti-SOX11$^{sc\text{-}17347}$. Results are summarized in Table 1.

Briefly, all 9 cyclin D1-negative MCLs were positive with both monoclonal antibodies. Among cyclin D1-positive MCL cases, 20/27 (74%) were positive with anti-SOX11$^{143}$ (Fig. 2) and 20/23 (84%) with anti-SOX11$^{MRQ\text{-}58}$, although with weaker staining (Table 2). None of the non-MCL cases was positive using the anti-SOX$^{MRQ\text{-}58}$. Nevertheless, SOX11 expression was observed in 31/173 (18%) non-MCL cases using anti-SOX11$^{143}$ and in 10/145 (7%) non-MCL cases using anti-SOX11$^{sc\text{-}17347}$. Two DLBCL cases were positive with anti-SOX11$^{143}$ (Fig. 3), and one of them was also positive with anti-SOX11$^{sc\text{-}17347}$, although with weaker expression levels. None of these 2 cases has SOX11 mRNA expression by qRT-PCR. Moreover, we observed a weak nuclear staining in follicular dendritic cells (FDC) and in the cells of high endothelial venules with the anti-SOX11$^{143}$, most evident in angioimmunoblastic T-cell lymphoma.

Among the 59 previously published cases stained with the polyclonal anti-SOX11$^{HPA000536}$,[4] we identified 4 discordant cases (7%). All these cases were Burkitt lymphoma (BL). Three previously reported negative cases turned to be positive with anti-SOX11$^{143}$, and one of

**TABLE 2.** Protein and mRNA Levels of SOX11 Expression by IHC and qRT-PCR on a Series of Cyclin D1-positive MCL

| Case | SOX11$^{143}$ | SOX11$^{142}$ | SOX11$^{MRQ58}$ | SOX11$^{sc\text{-}17347}$ | qRT-PCR |
|---|---|---|---|---|---|
| 1 | – | – | – | – | 0.03 |
| 2 | – | – | – | – | 0.79 |
| 3 | – | – | – | – | 1.21 |
| 4 | + | – | + | + | 3.53 |
| 5 | – | + | – | – | 3.88 |
| 6 | + | + | + | + | 9.57 |
| 7 | + | + | + | + | 9.97 |
| 8 | + | + | + | + | 13.21 |
| 9 | + | + | + | + | 13.32 |
| 10 | + | + | + | + | 14.87 |
| 11 | + | + | + | NP | 18.36 |
| 12 | + | + | + | + | 19.56 |
| 13 | + | + | + | + | 21.03 |
| 14 | + | + | + | + | 23.80 |
| 15 | + | + | + | + | 24.32 |
| 16 | + | + | + | + | 28.69 |
| 17 | + | + | + | + | 30.33 |
| 18 | + | + | + | + | 30.53 |
| 19 | + | + | + | + | 34.06 |
| 20 | + | + | + | + | 35.61 |
| 21 | + | + | + | + | 36.14 |
| 22 | + | + | + | + | 38.40 |
| 23 | + | + | + | + | 90.30 |

Cases with qRT-PCR values $\leq 1.21$ resulted as negative on IHC for SOX11, whereas cases resulted as SOX11 positive for qRT-PCR values $\geq 9.57$. qRT-PCR was performed in all cases on frozen material.
NP indicates not performed.

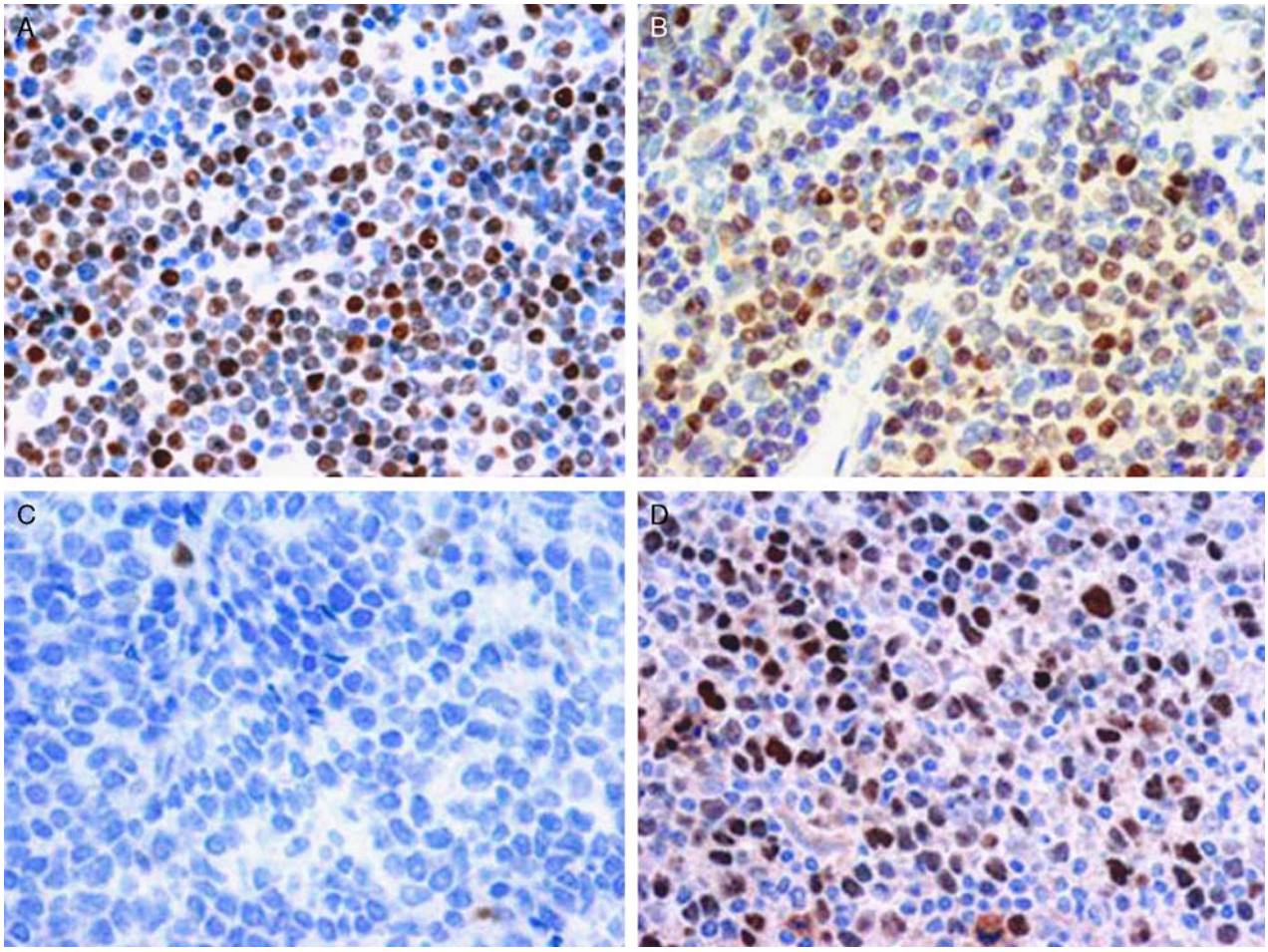


**FIGURE 2.** IHC analysis on cyclin D1-positive and cyclin D1-negative MCL. Detection of nuclear SOX11 expression in cyclin D1-negative (A and C) and in cyclin D1-positive cases (B and D). A and B, SOX11 staining using monoclonal anti-SOX11$^{143}$. C and D, cyclin D1 staining.

them was also positive with anti-SOX11$^{sc\text{-}17347}$. One previously reported positive BL did not shown expression with anti-SOX11$^{sc\text{-}17347}$. None of the discordant cases showed expression with anti-SOX11$^{MRQ\text{-}58}$.

## DISCUSSION

Here, we present the new mouse monoclonal anti-SOX11 antibodies, which show very good performance on FFPE tissue samples and are therefore suitable for routine diagnostic testing. *SOX11* represents an important diagnostic marker in MCL, as the presence of high levels of SOX11 mRNA and the protein in a nuclear localization are reported in most MCL cases, independent of their cyclin D1 status.[4,16] However, no study on the comparison between IHC detection of anti-SOX11 antibodies with SOX11 mRNA levels has been conducted so far. This might represent an important issue, as posttranscriptional regulation and posttranslational modifications can lead to changes in protein levels, and in routine diagnostic testing SOX11 is usually detected at the protein level by means of IHC. SOX proteins may be modified by ubiquitin and small ubiquitin-like modifier, which can affect their stability and subcellular localization.[22,23] On a series of 23 cyclin D1-positive MCL cases, we could show that both anti-SOX11$^{143}$ and anti-SOX11$^{MRQ\text{-}58}$ can detect SOX11 protein in all cases with qRT-PCR values $>9.57$. A similar correlation was obtained with 2 other antibodies, that is, anti-SOX11$^{HPA000536}$ and anti-SOX11$^{142}$, and minor differences were merely observed in 2 cases presenting with almost identical qRT-PCR values lying close to the IHC detection level. This suggests that changes in posttranscriptional and posttranslational modifications have a minor impact on the SOX11 cutoff value for protein detection on IHC.

The properties of anti-SOX11 antibodies were further studied on Western blot on extracts of cells overexpressing SOX11, as well as SOX4, as this represents the protein with highest homology belonging to the same subgroup, and were compared with those of anti-SOX11$^{142}$ and anti-SOX11$^{sc\text{-}17347}$. Anti-SOX11$^{143}$ recognized SOX11 and, to a far lesser extent, SOX4 similarly to anti-SOX11$^{142}$, which was raised against the same peptide of SOX11. However, the specificity and sensitivity of anti-SOX11$^{143}$ for SOX11

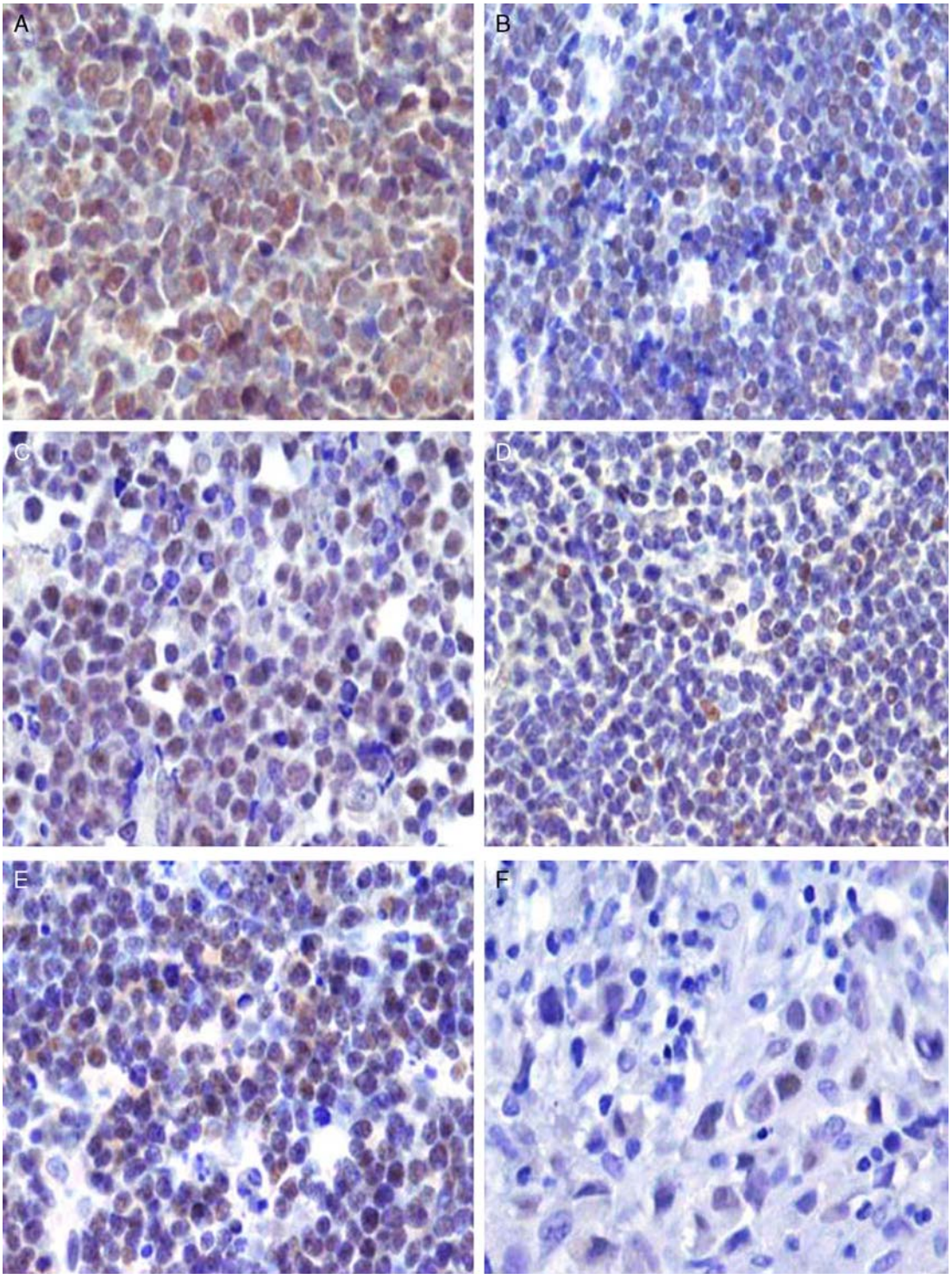


**FIGURE 3.** IHC results of SOX11 expression in lymphomas other than MCL using monoclonal anti-SOX11$^{143}$ antibody. Nuclear SOX11 expression in B-lymphoblastic lymphoma (A), T-lymphoblastic lymphoma (B), BL (C), T-cell prolymphocytic leukemia (D), DLBCL (E), and anaplastic large cell lymphoma, ALK negative (F).

were 4.0 and 8.1 times higher, respectively, when compared with the anti-SOX11$^{142}$ antibody. Anti-SOX11$^{sc\text{-}17347}$, which on Western blot assay gives very specific results, is 12.4 times less sensitive than anti-SOX11$^{143}$ antibody. The anti-SOX11$^{MRQ\text{-}58}$ did not showed cross-reactivity with SOX4.

In our series of 209 primary lymphoid tumors, immunostaining with anti-SOX11$^{143}$ gave results that are

**TABLE 3.** SOX11 IHC Expression Lymphomas in Previous Studies

| | n (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Diagnosis | SOX11 N-Term Dictor et al[6] | SOX11 C-Term Ek et al[5] | Monoclonal SOX11-C1 Nordström et al[17] | Polyclonal Sigma/Atlas Zeng et al[16] | Polyclonal Atlas Mozos et al[4] | Polyclonal Atlas Chen et al[7] | Polyclonal Atlas Ek et al[5] |
| B-LBL | 8/9 (89) | 3/5 (60) | 0/2 | 4/5* (80) | 1/1 (100) | | |
| T-LBL | 10/10 (100) | 4/5 (80) | 0/4 | | 5/5 (100) | | |
| CLL | 0/6 | | 0/14 | 0/5 | 0/12 | 0/50 | 0/47 |
| HCL | 6/12 (50) | 2/3 (67) | 0/7 | | | 5/10 (50) | |
| LPL | 0/1 | | | 0/5 | | | |
| PCM | 0/9 | 0/1 | | | 0/2 | 0/30 | |
| MZL | 0/16 | | | 0/5 | 0/20 | 0/12 | |
| FL | 0/4 | | 0/15 | 0/10 | 0/22 | 0/22 | 0/47 |
| MCL CCND1$^+$ | 18/23 (78) | | 15/16 (94) | 30/30 (100) | 50/54 (93) | 54/57 (95) | 26/28 (93) |
| MCL CCND1$^-$ | | | | 5/5 (100) | 12/12 (100) | | 1/1 (100) |
| DLBCL | 0/31 | | | 0/75 | 0/63 | 0/30 | 2/30 (7) |
| BL | 7/14 (50) | 3/4 (75) | 2/4 (50) | 1/5 (20) | 2/8 (25) | | |
| BCLU | 0/6 | | | | | | |
| T-PLL | | | | | 2/3 (67) | | |
| T-LGL | 0/1 | | | | | | |
| Extranodal | | | | | | | |
| NK/T-cell | 0/4 | | | | 0/3 | | |
| EATL | 0/2 | | | | | | |
| HSTL | 0/1 | | | | 0/3 | | |
| MF | 0/1 | | | | | | |
| PTCL, NOS | 0/4 | | | | 0/15 | | |
| AITL | 0/3 | | | | 0/5 | | |
| ALCL ALK$^+$ | 0/4 | | | | 0/3 | | |
| ALCL ALK$^-$ | | | | | 0/3 | | |
| NLPHL | 0/2 | | | | 0/5 | | |
| cHL | 0/5 | | | | 1/36 (3) | | |

*These cases included B-LBL and T-LBL without distinction.

AITL indicates angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; BCLU, B-cell lymphoma unclassified with features intermediate between DLBCL and BL; B-LBL, B-lymphoblastic lymphoma; cHL, classical Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; EATL, enteropathy-associated T-cell lymphoma; FL, follicular lymphoma; HSTL, hepatosplenic T-cell lymphoma; LPL, lymphoplasmacytic lymphoma; MF, mycosis fungoide; MZL, marginal zone lymphoma; NLPHL, nodular lymphocyte predominant Hodgkin lymphoma; PCM, plasma cell myeloma; PTCL NOS, peripheral T-cell lymphoma not otherwise specified; T-LBL, T-lymphoblastic lymphoma; T-LGL, T-cell large granular lymphoma; T-PLL, T-cell prolymphocytic leukemia.

mostly consistent with those previously reported (Table 3).[4–7,16] A fraction of the 209 lymphomas presented herein was included in a previous study that used polyclonal anti-SOX11$^{HPA000536}$ and showed highly concordant results.[4] Most cases of this same series were additionally stained with the polyclonal anti-SOX11$^{sc\text{-}17347}$, and results were similar, apart from a higher number of negative cases with anti-SOX11$^{sc\text{-}17347}$, which might be explained by its lower sensitivity on Western blot. Two of 43 DLBCL cases in our series showed weak nuclear SOX11 expression, similarly to that reported in a previous study.[5,24] qRT-PCR of these 2 cases was performed, which resulted in the absence of SOX11 transcript. These cases were also negative for cyclin D1. The absence of these 2 markers excludes a pleomorphic variant of MCL. These data, together with the cross-reactivity of the antibody observed on Western blot assay for SOX4, suggest that in rare cases weak nuclear positivity might be due to the high homology of the 2 proteins.[25] Analysis of data derived from previously published gene expression profile studies showed that some DLBCLs may express SOX4 but are negative for SOX11.[26] The nuclear staining observed in FDC and endothelial cells of angioimmunoblastic T-cell lymphoma with monoclonal anti-SOX11$^{143}$ might also be due to a cross-reaction with SOX4; however, the same positivity was observed with the anti-SOX11$^{sc\text{-}17347}$, which does not recognize SOX4 on Western blot. This suggests that SOX11 could be expressed in these 2 cell populations. In this regard, SOX11 expression has been reported in FDC neoplasms[27] as well as in endothelial cells and FDC of MCL in situ.[28] To clarify this issue, additional studies are needed, which might reveal a potential role of SOX11 as a marker for these 2 cell populations and for tumors deriving from them. Importantly, the good performance of the monoclonal anti-SOX11$^{143}$ and anti-SOX11$^{MRQ\text{-}58}$ on FFPE tissue samples is further supported by the fact that none of the cases of our series presented with unspecific cytoplasmic staining, in contrast with previous observations with polyclonal antibodies.[4,5]

Some studies have reported SOX11 expression in cyclin D1-positive hairy cell leukemia (HCL),[6,7] which, together with data on transcription of SOX11 and cyclin D1 in B-cell lymphomas,[29] suggested a possible dependence between SOX11 and cyclin D1 expression. In our series, however, all 5 HCL cases (3 of them being cyclin D1 positive and 2 cyclin D1 negative) were negative for

anti-SOX11$^{143}$ and anti-SOX11$^{MRQ-58}$, which is consistent with both the recently published results obtained with another monoclonal anti-SOX11[17] and data on the absence of SOX11 mRNA transcript in HCL.[5] Only 1 HCL case showed nuclear staining with anti-SOX11$^{sc-17347}$; however, this was negative for cyclin D1. Similarly, SOX11 is absent in cyclin D1-positive plasma cell myelomas, some of which carry the same t(11;14) translocation as MCL.[4,6,7,30] In contrast, SOX11 is expressed in lymphomas without t(11;14), such as BL, T-lymphoblastic lymphoma, and B-lymphoblastic lymphoma.[4,6,16,17] These data suggest that the expressions of cyclin D1 and SOX11 are independent.

Apart from the translocations of the *CCND2* gene, few data are available on the pathogenesis of cyclin D1-negative MCL.[31] In this regard, SOX11 might play a role in the development of cyclin D1-negative MCL, as its expression was shown to be oncogenic promoting tumor growth in a mouse model and to block plasmacytic cell differentiation by forced induction of the direct target gene *PAX5*.[14] This might also explain the different behavior of SOX11-negative MCL, as these patients present more frequently with non-nodal leukemic disease and an indolent clinical course, even though contrasting results have been reported.[8,9,18,32]

In conclusion, our data support that SOX11 expression by IHC correlates well with its transcription level. Most importantly, we have assessed the performance of several SOX11 antibodies on FFPE tissue sections. At least, 2 monoclonal antibodies, anti-SOX11$^{143}$ and anti-SOX11$^{MRQ-58}$, are useful in recognize MCL in routine diagnostic tissues. Although anti-SOX11$^{MRQ-58}$ seems to be more specific for SOX11 recognition in Western blot, anti-SOX11$^{143}$ may be more sensitive in IHC.

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